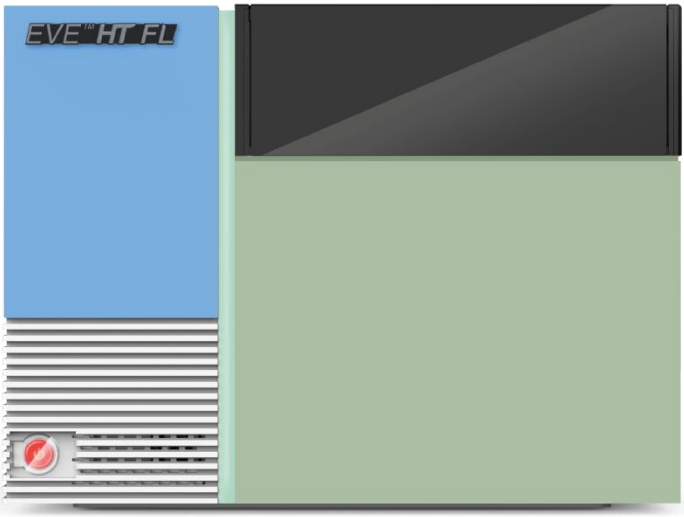
CE

EVE™ HT FL

User Manual



NanoEntek

EVE™ HT FL User Manual

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The information in this user manual is described as accurately as possible.

Firmware and software changes may occur without prior notification.

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V.0.3 SEP 2025

Table of Contents

Introduction

EVE™ HT FL is a high-throughput automated fluorescence cell counter. EVE™ HT FL can measure up to 48 samples simultaneously using disposable EVE™ HT FL Counting Plates. It takes only 3 minutes to measure all 48 samples with fast mode, and up to 20 minutes with accuracy mode. EVE™ HT FL requires 20 µL of samples to run measurements. EVE™ HT FL takes 2 fluorescence images (AO and DAPI channels) and optional bright field (BF) images. With BF images, EVE™ HT FL provides more accurate cell size histograms.

EVE™ HT FL can measure cell lines, primary cells, stem cells, and PBMCs. EVE™ HT FL has low user-to-user and instrument-to-instrument variations. EVE™ HT FL offers an optional “21 CFR part 11” module for data security and integrity which is compliant to FDA requirements.

Product Components

EVE™ HT FL consists of the following components.

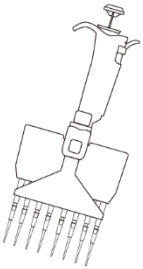
If any of the components are missing or damaged, please contact your local sales representative or send an email to **sales@nanoentek.com**.

EVE™ HT FL Instrument

1 EA

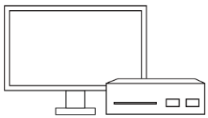
Multi pipette

1 EA



EVE™ HT FL desktop PC

1 SET



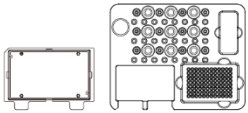
EVE™ HT FL counting kit

1 EA

EVE HT FL COUNTING KIT

EVE™ HT FL Accessories

Prepration plate (Optional)
QC plate (Optional)

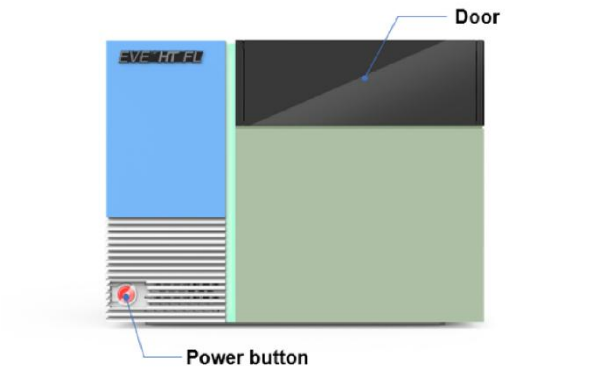


QC PLATE PREPARATION PLATE

EVE™ HT FL 21 CFR part 11 software (Optional)

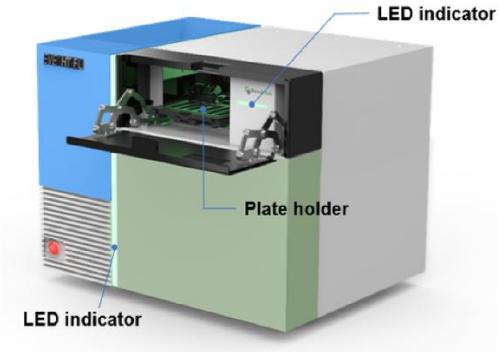
Product Description

Front view



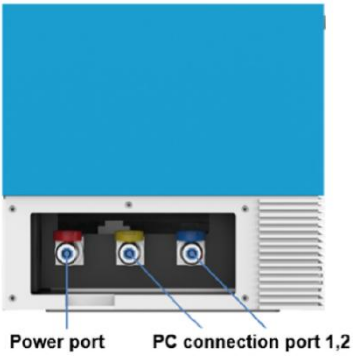
Part name	Description
Power button	Button to turn the instrument on or off
Door	Door to insert or retrieve EVE™ HT FL counting plate

Upper side view



Part name	Description
LED indicator	LED to indicate the state of EVE™ HT FL instrument
Plate holder	Holder to grip EVE™ HT FL counting plate tightly during measurements

Left side view



Part name	Description
Power port	Red port to supply power to EVE™ HT FL
PC connection ports	Yellow camera port and Blue port to communicate with EVE™ HT FL desktop PC

Installation

Environmental Requirements

For best performance, please see review the following recommendations to set up EVE™ HT FL:

- Use at room temperature.
- Do not expose instrument to direct sunlight.
- Do not expose instrument to continuous vibration.
- Do not expose instrument to intense magnetic or electromagnetic fields.
- Do not install instrument in high-humidity environment.
- Do not install instrument near corrosive gases or substances.
- Minimize contact with dust or airborne particles.
- Make sure to have at least 10 cm (4 inches) of free space around instrument for proper airflow.
- Avoid sharing an outlet and if possible, designate a wall outlet for EVE™ HT FL.

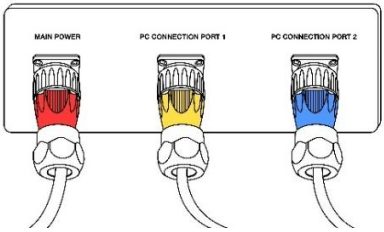
⚠ ***CAUTION***

If operating temperature is below 10 °C, wait for at least 10 minutes after turning on the instrument before use.

Installation

Installation and turning power on

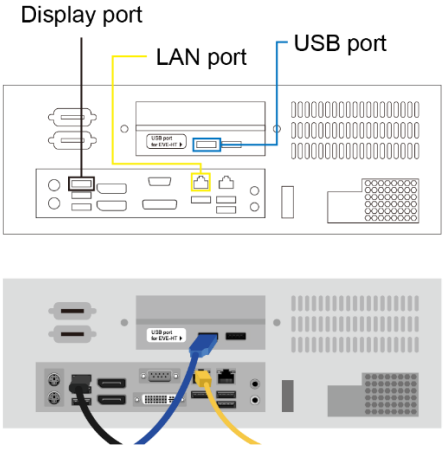
1. Find a flat space on a bench.
2. Open packages, put EVE™ HT FL instrument and EVE™ HT FL desktop PC on a flat space. Remove protective films.
3. Set up EVE™ HT FL desktop PC and monitor.
4. Unscrew 5 black bolts on the left side of EVE™ HT FL instrument to remove cover of side ports.
5. Connect three color coded connectors to matching ports.



6. Connect black cable to a wall outlet.
7. Connect yellow cable to the Ethernet port on the back of EVE™ HT FL desktop PC.

⚠ ***CAUTION***

- ***Make sure to connect yellow cable to the Ethernet port on a PCI board which is used for camera connection.***
- ***DO not connect yellow cable to the Ethernet port on the mother board which is used for internet connection***
- ***Please see the cartoon below.***



Installation

Installation and turning power on

8. Connect blue cable to a USB port on the back of EVE™ HT FL desktop PC.

⚠ ***CAUTION***

- ***Make sure to connect blue cable to one of the USB ports on a PCI board.***
- ***Do not connect blue cable to one of USB ports on the mother board.***

⚠ ***CAUTION***

- ***Do not tilt instrument too much when connecting the power cord.***
- ***Do not move instrument after connected to the power cord.***

9. Turn on EVE™ HT FL instrument and desktop PC.
10. Run EVE™ HT FL software.

⚠ ***CAUTION***

If an error code occurs during initialization, turn off both instrument and PC, and then restart both instrument and PC. If same error message appears repeatedly, contact your local distributor or sales@nanoentek.com.

Sample preparation

Recommended Actions

To obtain best results, follow these recommendations:

1. Handling sample

① Wear personal protective equipment while handling samples.
② Make sure to mix sample well at every step.
③ After loading prepared sample onto counting plate, wait for **2 minutes** to let cells settle down on the bottom surface.

2. Staining reagent

① Store the staining reagent at the appropriate temperature.
- AO/DAPI staining solution - in a refrigerator or on ice
- Trypan blue or Erythrosin B stain - at room temperature

3. EVE™ HT FL Counting Plate

① Keep counting plates in protective carriage case until use.
② Make sure to put counting plates on a clean surface.
③ Do not touch any other parts of counting plates except for the handles.
④ Make sure to fill entire well.
⑤ Do not tilt counting plate after loading samples.
⑥ Do not insert counting plate upside-down.
⑦ Make sure to push the plate all the way in.
⑧ Do not reuse those wells that have been filled up with samples. However, same plate can be used multiple times as long as there are unused wells.

4. Starting EVE™ HT FL software

① Make sure to turn on EVE™ HT FL instrument BEFORE starting EVE™ HT FL software.
② Make sure that door and plate holder are closed when starting EVE™ HT FL software.

Sample preparation

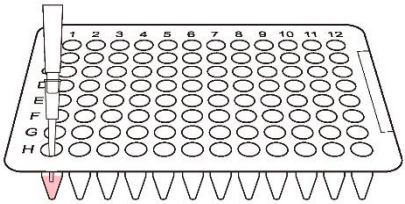
Staining samples

1. Prepare samples in growth media or PBS. Make sure that cells are well separated and suspended. If needed, vortex samples before starting staining procedure.

☛ ***NOTE***

EVE™ HT FL can analyze cell concentrations of 1 x 10^4 to 2 x 10^7 cells/mL.

2. If needed, concentrate or dilute samples.
3. Load **20 µL of well-mixed sample** into a mix well plate. ← Mix well plates (PCR plates) are included in EVE™ HT FL kit.

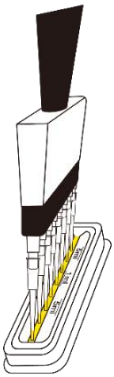


4. Dispense enough amount of **staining solution** to a reservoir. ← Disposable reservoirs are included in EVE™ HT FL kit.

☛ ***NOTE***

Use dispensed staining solution as soon as possible. It is not recommended to leave solution in a reservoir.

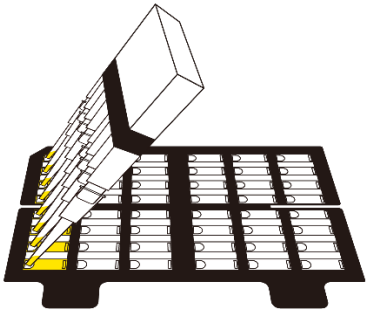
5. Add **20 µL of staining solution** to those wells that are loaded with cell samples using multi pipette. **Mix sample and staining solution well by pipetting up and down**. ← 8 channel pipette is included in EVE™ HT FL.



Sample preparation

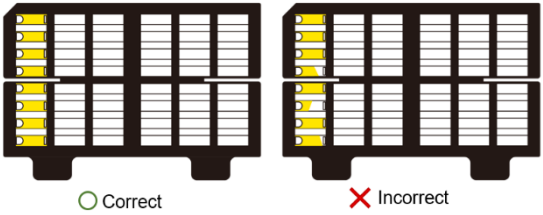
Staining samples

6. Load **20 µL of mixed samples** into EVE™ HT FL Counting plate.



☛ ***NOTE***

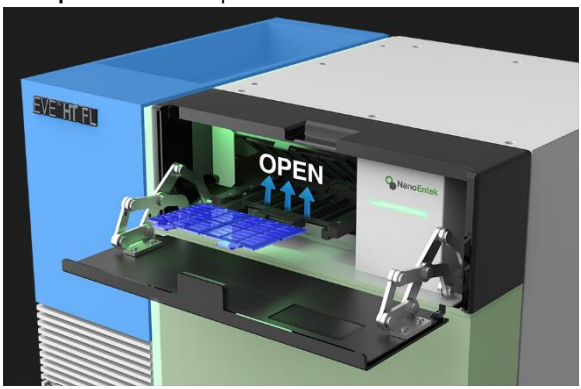
Examples of correctly loaded counting plate and incorrectly loaded counting plate



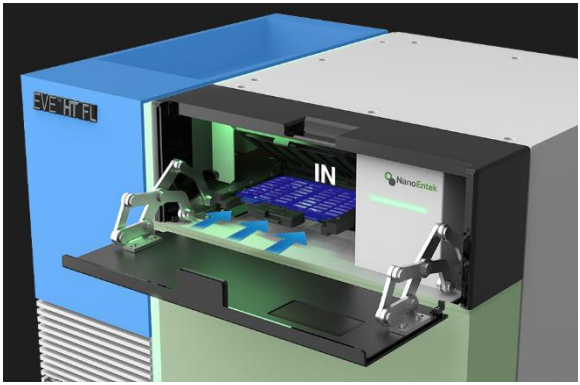
Sample preparation

Insert counting plate

1. Open black door in front of the instrument by pulling the door forward and find the black plate holder. Press the bar in the middle of the **plate holder** to open the holder.



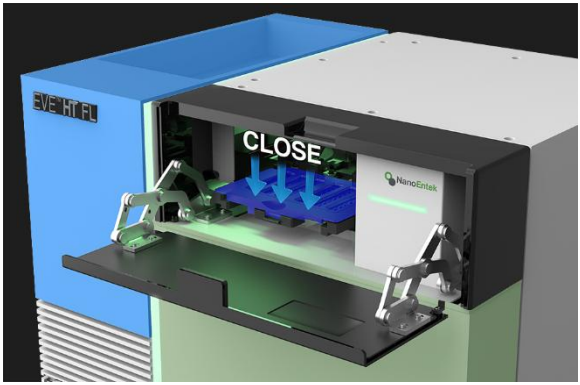
2. Insert **EVE™ HT FL Counting plate** loaded with samples into the plate holder.



Sample preparation

Insert counting plate

3. Close the **plate holder** by pressing the holder cap down until you hear “click” sound, and close the **door**.



⚠ ***CAUTION***

Allow samples to settle for ‘2 minutes’ after loading samples onto EVE™ HT FL Counting Plate. One can wait either before or after inserting plate.

⚠ ***CAUTION***

Make sure to put EVE™ HT FL Counting Plate all the way in.

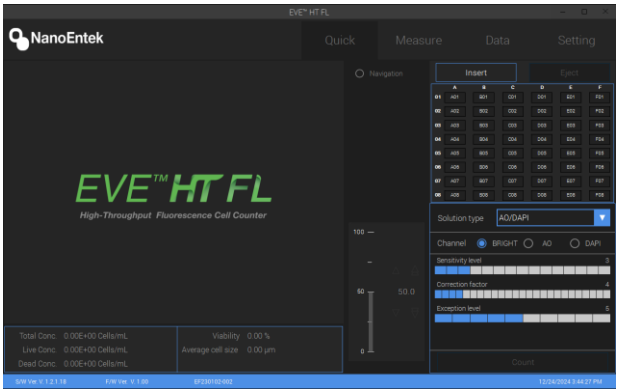
⚠ ***CAUTION***

Make sure to properly close plate holder cap and door before starting measurement.

Quick count

Quick count

The quick count function gives a quick measurement of what is being shown on live feed. One can use quick count function to determine whether sample needs further dilution or to adjust counting parameters.



1. Select the solution type.
2. To move the plate holder to imaging position, click the **'Insert'** button.
3. After counting plate is at imaging position, choose one of the wells loaded with samples.
4. Adjust focusing using up or down arrows. Please refer to example images below.

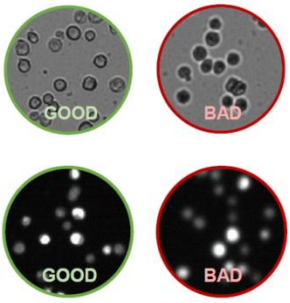
If there are more than one cell type, users will be asked to adjust focusing for each cell type. **Fine focusing** is also available. To use fine focusing, move mouse cursor on the preview window and turn mouse wheel while holding "ctrl" or "shift" key.

Quick count

Quick count

☛ ***Note:*** *Focus example*

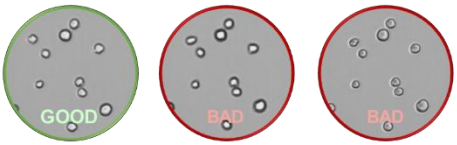
AO/DAPI



BRIGHT : The outline is clear, and the brightness inside the cells is the same as the background.

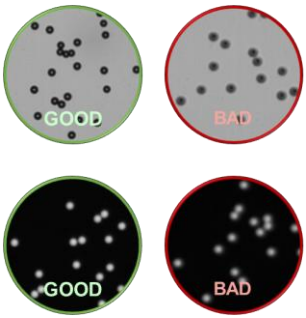
AO&DAPI : clear cell outline

Trypan blue or Erythrosin B



The outline is clear, and the brightness inside of live cells is brighter than the background.

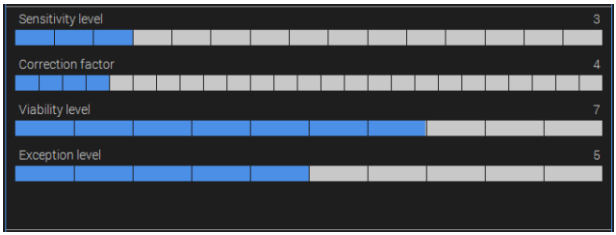
Test beads



The outline is clear in both the Bright and AO channels, with no blurriness.

Counting parameters

Counting parameters are used to deal with wide varieties of cell sizes and shapes. The description of each parameter is as follows.



1. BRIGHT channel

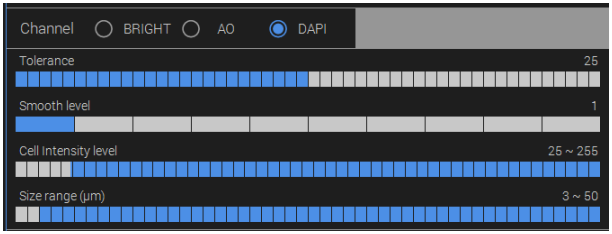
Sensitivity level : Decreasing this sensitivity parameter will make it easier to prevent debris from counting. (Caution: if the sensitivity level is too low, then some of the real cells may not be counted)

Correction factor : Decreasing this correction factor will make it easier to detect cells which are slightly darker than the background. When cells look very transparent, you can decrease this correction factor to pick up vague objects.

Viability level : Increasing this viability level will make it easier to detect marginally dark cells as dead cells. As you have tried already, increasing this level will increase the dead cell count.

☛ ***Note:*** *Viability level is available when using Trypan blue or Erythrosin B*

Exception level : Decreasing this exception level will make it easier to detect small objects.



2. AO or DAPI channel

(parameters need to be set for each channel)

Tolerance: Tolerance defines how to count dividing cells. Decreasing this parameter will allow counting cells in the middle of cell division or adjacent cells as individual cells. While increasing it will make large cells or aggregated cells to be counted as one cell.

Counting parameters

Smooth level: The smoothness level controls the smoothness of images. When cells are large and intracellular organelles are distinct enough to be counted as individual cells, you can increase this level to smooth out such distinctive textures.
Cell intensity level: Cell intensity level works similar to intensity gating. When cells are very dim or too bright, you can change the range of intensity levels of cells to be counted. Here, you can choose both minimum and maximum values.
Size range: Size range refers to the range of fluorescent cell sizes. This allows you to determine the size range and exclude small debris from the count.

To quickly test selected parameter sets, click the **'Count'** button and find results.

The following table provides examples of recommended parameters for commonly used cell lines. These are recommendations and can be further adjusted by users.

☛ ***Note:*** *Counting PBMCs or small diameter cells with Trypan blue or Erythrosin B is not recommended.*

☛ ***Note:*** *Using AO/DAPI solution is recommended for accurate cell count and viability analysis.*

AO/DAPI staining solution

	Cell size	Bright		
		Sensitivity level	Correction factor	Exception level
PBMC	2-80	3	4	5
CHO	5-80	3	4	5
Jurkat	5-80	3	4	5
U2OS	7-80	3	4	5
HeLa	8-80	3	4	5
HepG2	9-80	3	4	5

	AO				DAPI			
	Tolerance	Smooth level	Cell Intensity level	Size range	Tolerance	Smooth level	Cell Intensity level	Size range
PBMC	5	1	25-255	2-50	5	1	25-255	2-50
CHO	25	3	25-255	3-50	25	3	25-255	3-50
Jurkat	5	1	25-255	3-50	5	1	25-255	3-50
U2OS	5	1	25-255	3-50	5	1	25-255	3-50
HeLa	25	1	25-255	3-50	25	1	25-255	3-50
HepG2	25	3	25-255	3-50	25	3	25-255	3-50

Quick count

Counting parameters

Trypan blue stain

	Cell size	Sensitivity level	Correction factor	Viability level	Exception level
CHO	5-80	3	4	7	5
U2OS	7-80	3	4	7	5
HeLa	8-80	3	4	7	5

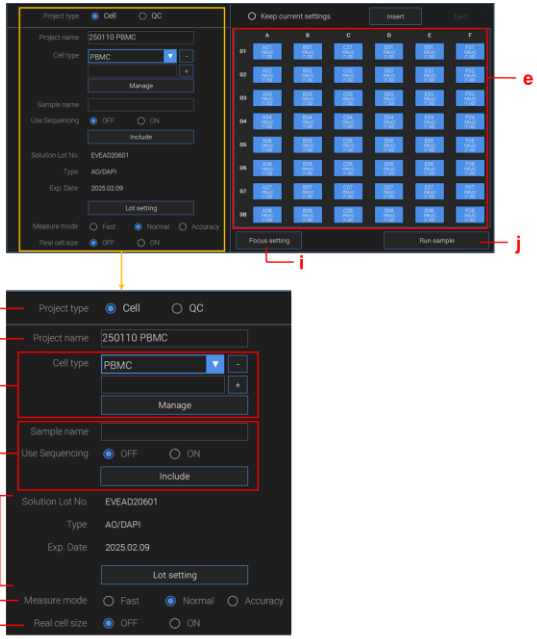
Erythrosin B stain

	Cell size	Sensitivity level	Correction factor	Viability level	Exception level
CHO	5-80	3	4	5	5
U2OS	7-80	3	4	5	5
HeLa	8-80	3	4	5	5

Measure

Measure setting

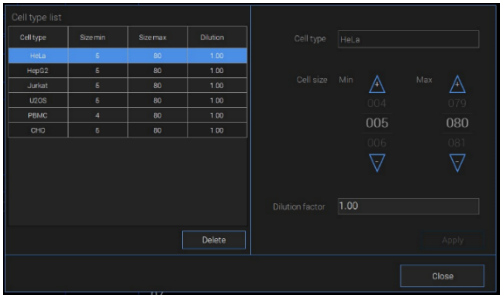
In the **Measure** tab, follow the steps described below to run measurements.



a. **Project type**: select “Cell” to run cell samples.
Select “QC” to run quality control samples. Please refer to ‘**QC**’ for details.

b. **Project name**: enter a project name.

c. **Cell type**: select one of the existing cell types or add a new one. Click **‘Manage’** to review and revise the preset size parameters.



Measure setting

d. **Define plate map**: repeat the following steps for all the wells to be measured.

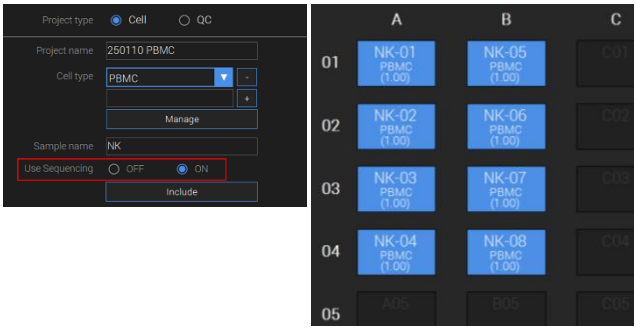
① After selecting a cell type, enter sample name. This field can be left blank.

② Select all the wells having this cell type in the plate map (**e**).

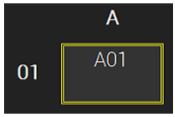
③ Click **'Include'** button.

If samples are replicates, one can group wells together by placing mouse cursor in the plate map, clicking the right mouse button and choosing "Include as a Group".

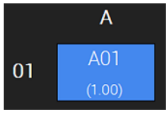
After entering the sample name, set **'Use Sequencing'** to **'ON',** and click **'Include'**, a sequential number is assigned to the sample name.



e. **Plate map**: click individual wells or click-and-drag group of wells to select wells.

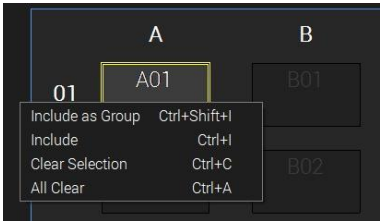


Selected well
(not included yet)



Included well

If you right-click on the well section, you can use functions such as Include and Exclude.



Measure

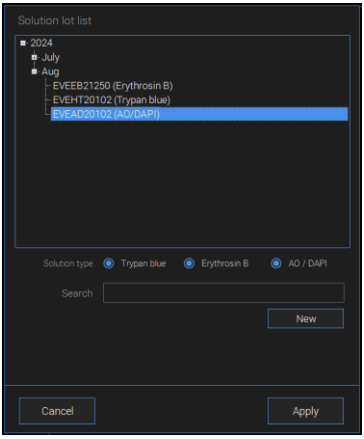
Measure setting

If you right-click outside the well, you can import or export plate maps.

If you select Format, you can download an Excel file with a blank format. You can enter information such as sample name and cell type directly into this Excel file before running measurements and then import predefined format.

☛ ***Note:*** *If the parameters in the Excel file do not match the values of the* ***Cell type****, the values from the Excel file will take priority, and the* ***Cell type*** *parameters will be updated accordingly.*

f. **Solution**: one can confirm the information about reagent solutions.
Click “Lot setting” to change or add new solution.



g. **Measure Mode**: choose “**Fast**” to take 1 image. If it is “**Normal**”, 4 images per well will be taken. A total of 15 images are taken if “**Accuracy**” is chosen.

☛ ***Note:*** *Only taking 4 images per well is available when using Trypan blue or Erythrosin B.*

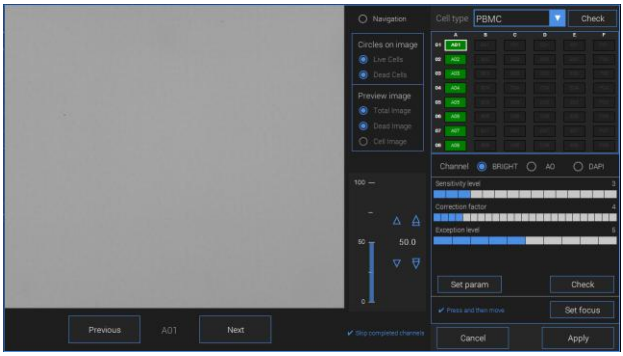
h. **Real Cell Size**: choose “**ON**” to take BR images which will give cell size estimates. (Available only when using AO/DAPI solution.)

i. **Focus setting**: see next page for details.

j. **Run sample**: click this button to start measurements.

Measure

Focus setting



When you press the focus setting button, the window below will appear. You can fine-tune the focus and parameters for each cell type here.

Navigation	Navigation on/off.
Circles	Circle (Live Cells / Dead Cells) on/off.
Preview image	Image (Total / Dead / Cell) on/off. *- Available only when using AO/DAPI solution.*
Previous/Next	Previous/Next move buttons.
Cell type	Select the cell type to set focus on and check it.
Channel	Select a channel to preview (BRIGHT / AO / DAPI) *- Available only when using AO/DAPI solution.*
Set param	Set current parameters.
Check	Count the cells on the current screen using the Quick count method.
Set focus	Apply the focus value.
Press and then move	After applying the focus setting, move to the next channel.
Skip completed channels	Channels that have been focused will be skipped.
Cancel/Apply	Cancel or apply current focus settings.

Measure

Focus setting

Follow the steps below to set focus.

1. Preview
- Zoom In and Out: put mouse cursor on the preview and turn mouse wheel.
- Select channel: Choose between BRIGHT, AO, or DAPI.

2. Plate map: Wells marked with BLUE indicate the wells of which focuses have not been set. When focuses are set, wells will be marked GREEN.
- Only the focus of the first well of each cell type needs to be adjusted.

3. Adjust focus: There are multiple ways to adjust focuses.
① Click single (fine steps) or double (coarse steps) arrows.
② Put mouse cursor on the preview, hold “ctrl” or “shift” key and turn mouse wheel.
Examples of good and bad focuses can be found on page 18.
③ Click **‘Check’** to see counting results of the current view.

4. Save focus: Click **‘Set focus’** to save focuses.
- If there are more than 1 cell type, preview will automatically move to the first well of the next cell type.

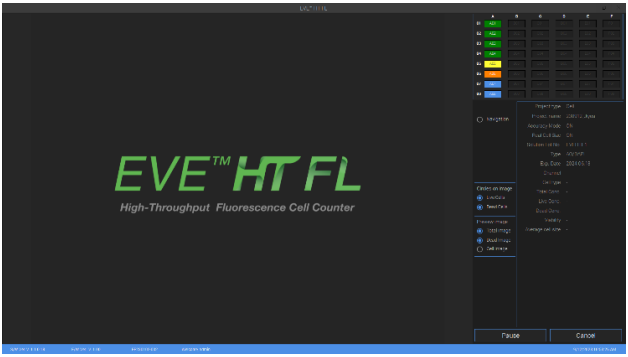
5. Image analysis parameters: Determine parameters for each cell type. Please see pages 19-21 for details.

6. Set param: If a parameter has been changed, press this button to save.

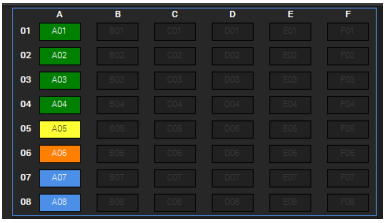
7. Finish focus setting: After saving focuses of all wells, click **‘Apply’** button.

Measurements and Calculations

After clicking **'Run sample'**, the following window appears, and the measurement will start following the plate map information entered previously.



You can check the current measurement status on the plate map in the upper right.



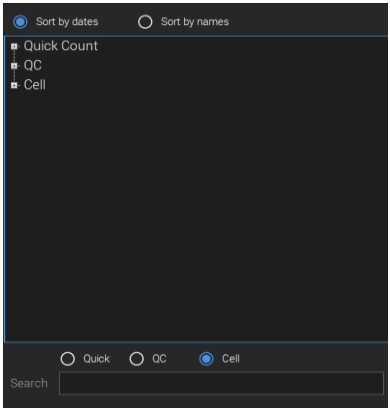
Blue: To be measured.
Orange: Image acquisition is in process.
Yellow: Image acquisition is done but image analysis is in process.
Green: Measurement has been completed.

If one clicks one of the GREEN wells, one can check images and results of the well.

Data

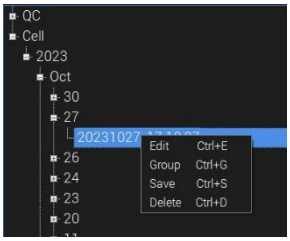
Data list

You can check the data list and results by clicking the **Data** tab.



View type	It can be viewed by two criteria, date or name.
Quick Count	Data list taken from the Quick menu.
QC	Data list taken from the QC mode (Project type).
Cell	Data list taken from the Cell mode (Project type).
Search	Search the data in each section.

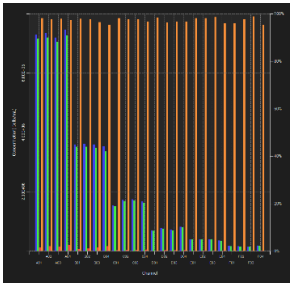
Right-click on each project to find sub-menu.

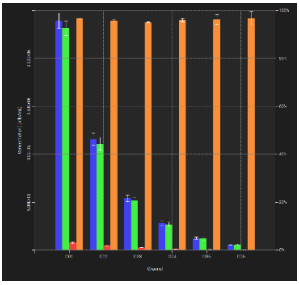


Edit	Rename the project name. Set the Size gating and dilution factor. Acceptance range is only available in QC data.
Group	To edit group setting, select **Group**.
Save	To save project, select **Save**. Select the data type and data path in the pop-up window.
Delete	To delete project, select **Delete**.

Graph and Table

Data measured with Quick Count, results will only include images and counting summary.
Data measured with QC as a project type, results will show whether results are consistent. (See Quality Control chapter for details.)
Data measured with Cell as a project type, results will appear in graph and table form.





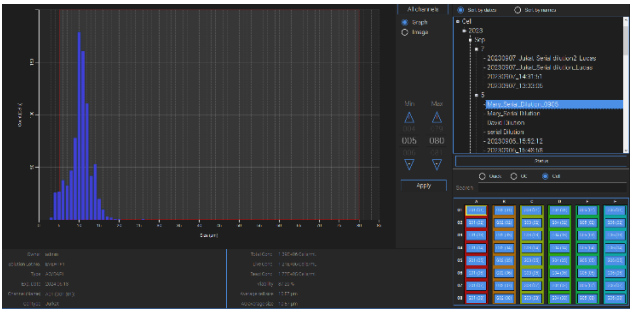
The graph shows total counts (BLUE), live cell counts (GREEN), dead cell counts (RED), and viabilities (ORANGE). You can hide any of these graphs by unchecking boxes below the graph. Grouped data will be displayed as a single graph.

Channel	Name	Total Conc.	Live Conc.	Dead Conc.	Viability (%)	Average Size (µm)
Group	G01	2.39E+06	2.31E+06	7.69E+04	96.79	11.36
	Std dev	7.64E+04	7.41E+04	8.47E+03	0.33	0.10
	Std err	3.82E+04	3.70E+04	4.24E+03	0.16	0.05
	CV	3.19	3.20	11.02	0.34	0.90
Group	G02	1.16E+06	1.11E+06	4.90E+04	95.75	11.37
	Std dev	6.31E+04	6.49E+04	6.67E+03	0.67	0.08
	Std err	3.15E+04	3.25E+04	3.33E+03	0.33	0.04
	CV	5.45	5.85	13.60	0.70	0.67
Group	G03	5.40E+05	5.14E+05	2.61E+04	95.17	11.30
	Std dev	3.64E+04	3.49E+04	1.69E+03	0.14	0.15
	Std err	1.82E+04	1.74E+04	8.46E+02	0.07	0.08
	CV	6.74	6.79	6.49	0.15	1.33
D05	D05	2.65E+05	2.56E+05	8.84E+03	96.67	11.55
D06	D06	2.97E+05	2.86E+05	1.06E+04	96.43	11.54
D07	D07	3.04E+05	2.92E+05	1.24E+04	95.93	11.42
D08	D08	2.46E+05	2.33E+05	1.24E+04	94.96	11.39
E05	E05	1.08E+05	1.02E+05	5.30E+03	95.08	11.42
E06	E06	1.24E+05	1.20E+05	3.53E+03	97.14	11.87
E07	E07	1.40E+05	1.38E+05	1.77E+03	98.73	11.47
E08	E08	1.22E+05	1.15E+05	7.07E+03	94.20	11.55
F05	F05	6,01E+04	5,83E+04	1,77E+03	97.06	10.94
F06	F06	6.01E+04	6.01E+04	0.00E+00	100.00	11.70
F07	F07	4.95E+04	4.59E+04	3.53E+03	92.86	11.10
F08	F08	5.83E+04	5.65E+04	1.77E+03	96.97	11.46

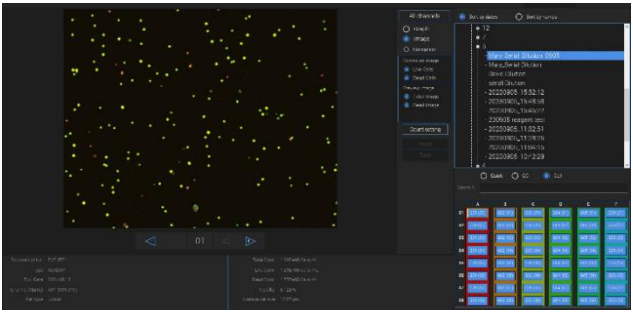
Data for each well (or group) is organized and displayed in a table as shown above. When you click on one of the column names, results will be sorted in ascending or descending order of the selected column (Group data cannot be sorted). For grouped data, the standard deviation, standard error, and CV of each group are automatically calculated and displayed.

Individual well results

When one well is selected, cell counts (Total, Live and Dead cell concentrations), viability, and average cell size will be shown below cell size histogram. You can change minimum or maximum cell sizes to be included in the results.

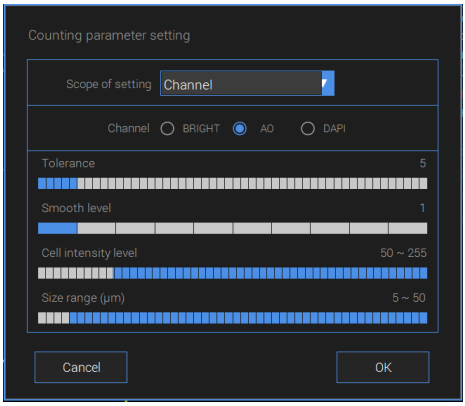


By selecting **'Image'**, you can review raw images. If **'Real cell size'** has been selected when using AO/DAPI solution, bright channel images will also be shown.



Individual well results

If you click **'count setting'**, you can change counting parameters in the current data. Refer to page 19-21 for a detailed explanation of parameters. Parameter values can be applied simultaneously to Channel, Cell type, Group, or Project.



By right-clicking and dragging the image, you can select cells individually as shown below to manually assign those objects in the selected area to either live or dead cells, or debris.

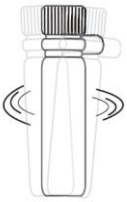
Quality control

QC bead preparation

Bead counting can be used as a way to evaluate whether instrument is in good condition.

☛ ***Note:*** *Ordering information is on page 60-61.*

1. Shake bottle vigorously or vortex briefly for 5 seconds before use.

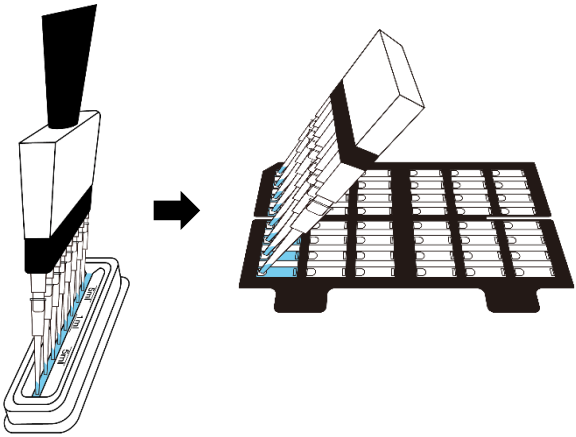


[Shake bottle vigorously]

[Vortexing]

2. Transfer **20 µL of test beads** to the Mix Well Plate.
For **FL test beads**, add **20 µL of PBS**.
For **BR test beads**, add **20 µL of Trypan blue or Erythrosin B**.
Mix well by pipetting up and down.

3. Take 20 µL of diluted test beads using a multi-pipette and load onto a counting plate.



QC bead Preparation

4. Open EVE™ HT FL door and open plate holder.

5. Insert counting plate loaded with sample into the plate holder.

6. Close the plate holder cap and close the door.

⚠ ***CAUTION***
Allow beads to settle for '1 minute' before starting measurements.

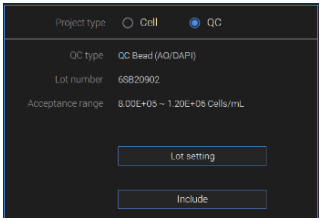
⚠ ***CAUTION***
Make sure to push the plate all the way in.

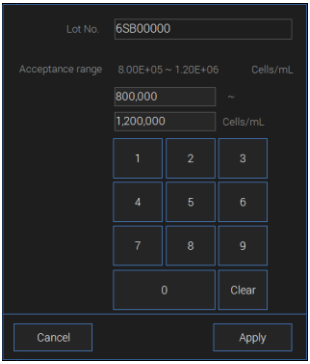
⚠ ***CAUTION***
Make sure door and plate holder are properly closed.

QC bead Run

After loading QC beads onto a counting plate, proceed with the settings in the Measure tab as follows.

1. Select **QC** as a project type and click '**Lot setting**' when using a new lot or for the first time.



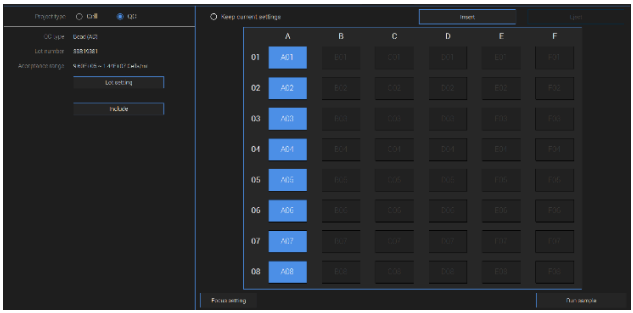


2. Click **'New'** button, enter lot number and acceptance range as written in the label of test beads, and then click **'Apply'** to save.

QC bead Run

3. Select the lot you use and click **‘Apply’** button.

4. Select the wells loaded with QC samples. For multiple selection, left click and drag.



5. Click **‘Include’** button or select ‘Include’ option from sub-menu by right clicking. Make sure selected wells are displayed in blue as shown.

6. Click **‘Focus Setting’** to adjust focus.

7. Adjust focuses on both BRIGHT and AO channels for FL test beads, or on BRIGHT only for BR test beads.

☛ ***Note:*** *Focusing for DAPI channel will be automatically updated based on AO focus position.*

☛ ***Note:*** *For information on how to focus, see page 18.*

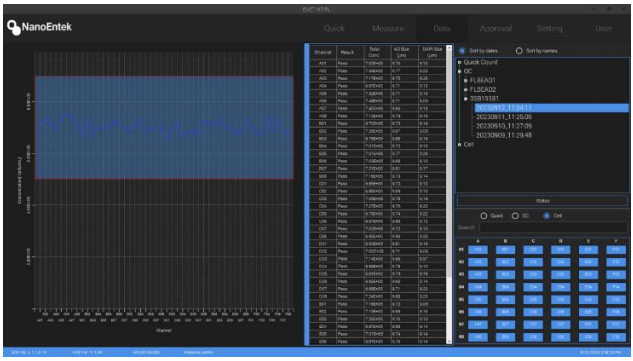
8. After focusing, click **‘Set focus’** button to save and then click **‘Apply’** button.

9. Click **‘Run sample’** button to start measurement.

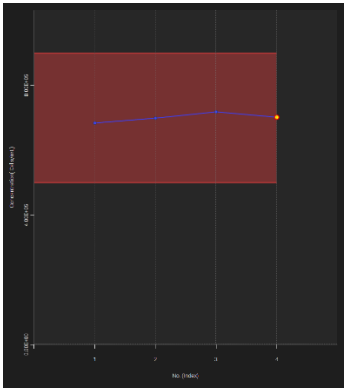
Quality control

QC bead Run

After measurement is completed, select data from the data list and check the dot graph as shown below. One can check total counts of beads, and sizes of beads.



If you click on a bead lot number in the data list, you can see the results of all previous measurements of the selected lot.



Quality control

QC plate Preparation

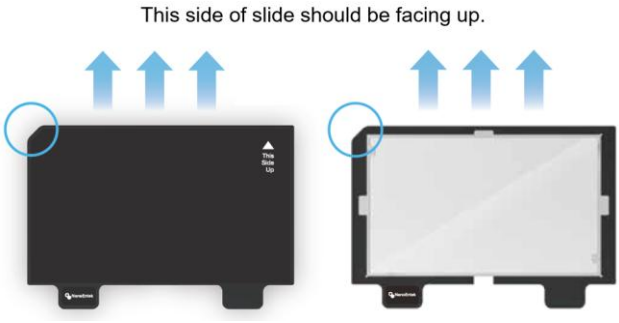
This is for quality control using QC plate. Follow the instruction below only if necessary.

1. Prepare the QC Plate.

☛ ***Note:*** *Ordering information is on page 60-61.*

2. Open the EVE™ HT FL door and open plate holder.

3. Insert the **QC Plate** into the plate holder.



4. Close the plate holder cap and close the door.

⚠ ***CAUTION***

Make sure to push the plate all the way in.

⚠ ***CAUTION***

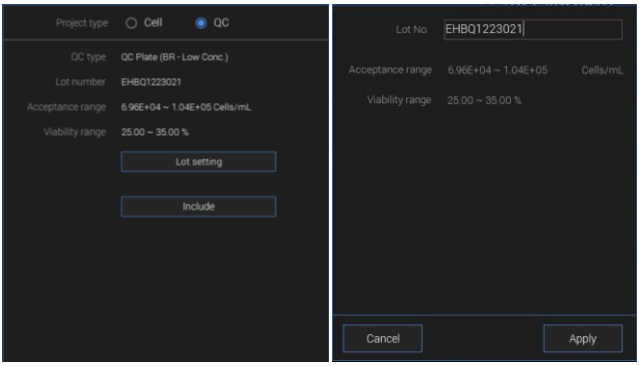
Make sure door and plate holder are properly closed.

Quality control

QC plate Run

After insert the QC plate into the instrument, proceed with the settings in the Measure tab as follows.

1. Select **QC** as a project type and click '**Lot setting**' when using a new lot or for the first time.



2. Click **'New'** button, enter lot number and check the acceptance range as written on the plastic package label of QC plate, and then click **'Apply'** to save.

3. Select the lot you use and click **'Apply'** button.

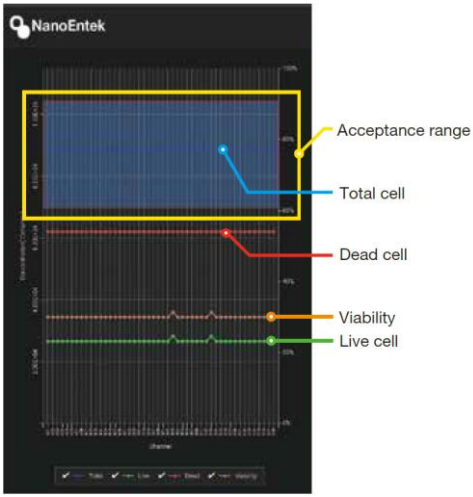
4. Select all 48 channels. For multiple selection, left-click and drag.

QC plate Run

5. Click **'Include'** button or select 'Include' option from sub-menu by right clicking. Make sure selected wells are displayed in blue as shown.

6. Click **'Run sample'** button to start measurement.

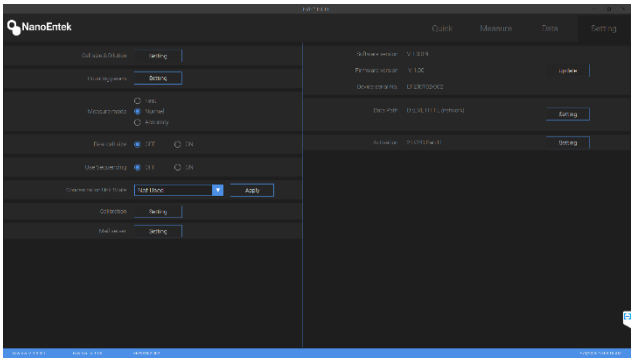
After measurement is completed, select data from the data list and check the dot graph as shown below.



If you click on a plate lot number in the data list, you can see the results of all previous measurements of the selected lot.

Setting

Setting tab



In **Setting**, you can set default values for parameters and dilution factors. These value sets will be used when a cell type is not specified or a new cell type is created.

Cell size & Dilution	Cell size and dilution factor of sample
Counting parameter	Parameter values for each BRIGHT, AO, and DAPI channel
Measure mode	Default analysis mode when using AO/DAPI staining solution
Real cell size	Default setting when using AO/DAPI staining solution
Use Sequencing	Default setting for assigning sequential number after the sample name
Concentration Unit Scale	Fixes the display of concentration values in exponential format (1.00E+01 to 1.00E+09). **Not applied to QC data*
Calibration	Calibration of brightness Calibration of the instrument image background level (Only for Trypan blue or Erythrosin B stain)
Mail server	**Do not change Mail settings.*
SW, HW information	Information about installed software and hardware
Data path	Path where measurement images and data are saved
21 CFR part 11 activation	Activate an optional program that complies with 21 CFR part 11

☛ ***Note**: Mix ratio (sample 20 µL + reagent 20 µL) is already applied so do not apply to dilution factor.*

Calibration

Backgrounds that are overly bright or dark may affect the results. Do the Background calibration when using Trypan blue or Erythrosin B with a new lot number.

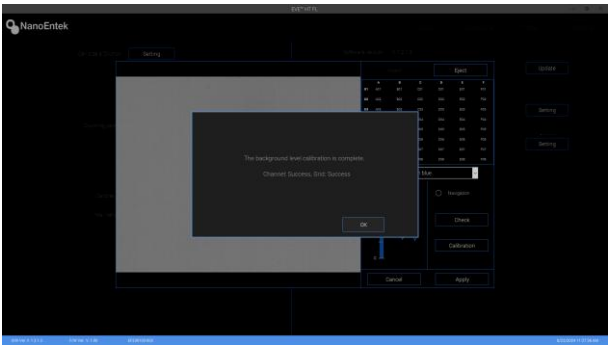
1. Calibration sample preparation
   - ① Mix **20 µL of culture media** and **20 µL of Trypan blue or Erythrosin B** thoroughly.
   - ② Load **20 µL of the mixture** into one channel in an EVE™ HT FL Counting Plate.
   - ③ Insert the counting plate into the plate holder of the instrument.

2. Calibration setting
   - ① Select '**Setting'** tab.
   - ② Click **Calibration 'Setting'** button.

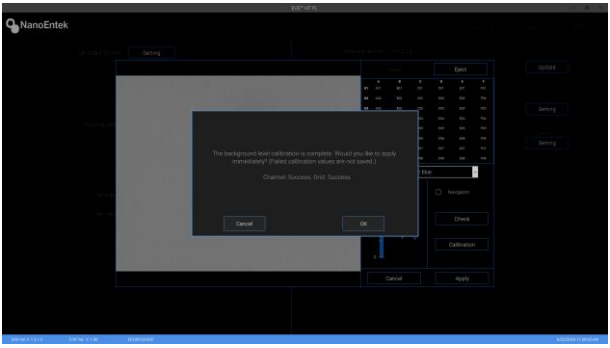
   - ③ Select the solution type for calibration
   - ④ Click **'Insert'** button.

Calibration

⑤ Select the wells loaded with the mixture.



⑥ Click **‘Check’** button.



⑦ Click **‘Calibration’** button.

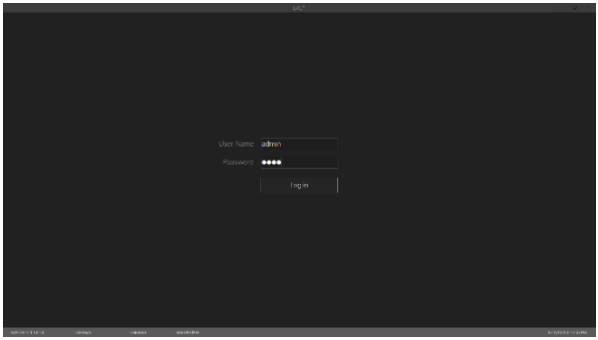
⑧ After finish the calibration, click **‘OK’** and **‘Apply’** button.

21 CFR part 11

The Food and Drug Administration (FDA) of the United States has established regulations on electronic records and electronic signatures (ERES) in Title 21 of the Code of Federal Regulations, specifically 21 CFR Part 11. EVE HT FL offers 21 CFR part 11 compliance program for cGMP facilities as an option. Please contact technical support to add this option.

Log In

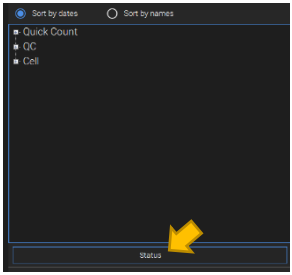
When activating 21 CFR Part 11, the user must log in on the first screen. Log in from login screen. (default ID/PW : admin/0000)

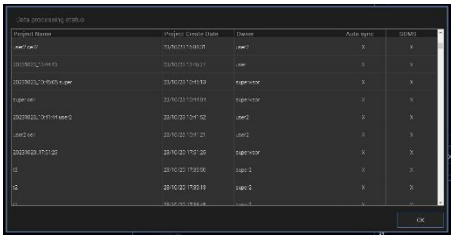


☛ ***Note**: Log out is in the User tab.*

Data status

Users can check data status upon 21 CFR Part 11 activation.

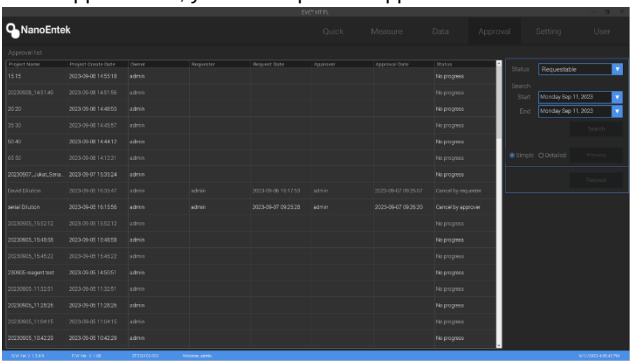




21 CFR part 11

Approval

In the approval tab, you can request or approve data.



You can check the report file for the data by double-clicking the data or clicking the Preview button.

Data list is divided into four status (Requestable, Requesting, Approvable, Approved) types depending on the approval status.

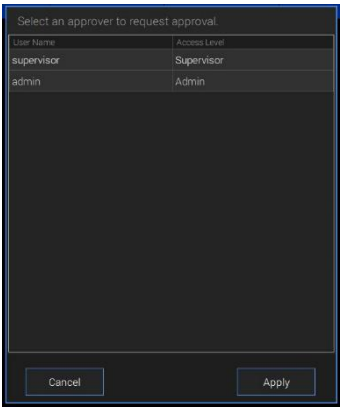
1. **Requestable**

The list shows data that can be requested for approval.

① Select the data to get approval.

② Click the **'Request'** button.

③ Enter the requester's password.



④ Select an approver to request approval.

⑤ Click the **'Apply'** button.

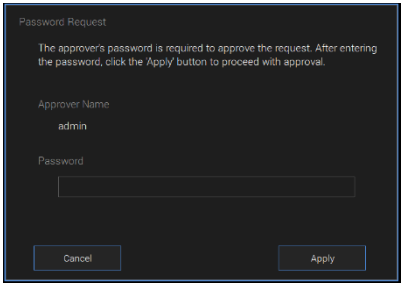
Approval

2. **Requesting**

The requesting data are displayed. It is possible to **'Cancel'** the request for approval. The approver can approve the request in Requesting tab. The approval no need to log in to the approval ID.

① Select the Data in list

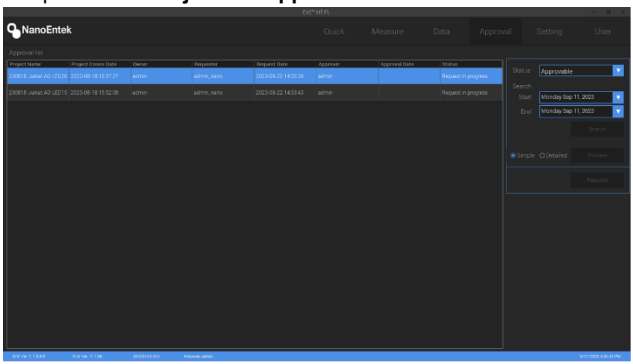
② Click the **'Approval'**



③ Enter the approver's password.

3. **Approvable**

The approvable data are displayed on this tab.
It is possible to **'Reject'** or **'Approval'**.



4. **Approved**

The approved data is listed.
It is possible to **'Export'** the Approved data.

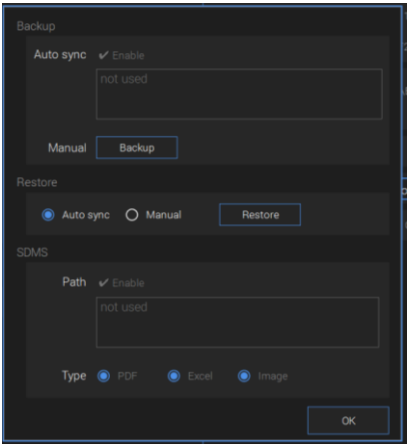
21 CFR part 11

Setting

In the 21 CFR part 11 program, several functions are added to the setting tab.

1. Backup & Restore

① Click the Backup & Restore **'Setting'** button.



② To enable automatic backup, click the Auto sync **'Enable'**, set the backup data path.

③ Click the Manual **'Backup'** button, save the backup data at the current point in time.

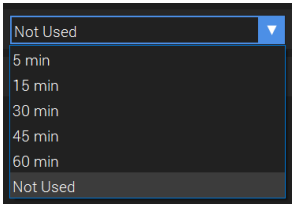
④ The Restore function provides two options. You can back up based on the backup data saved by Auto sync or the backup data saved by Manual.

⑤ Click the SDMS Path **'Enable'**, set the SDMS data path.

⑥ Select the desired type of SDMS data.

2. Auto logout

① Click the '▽' button.



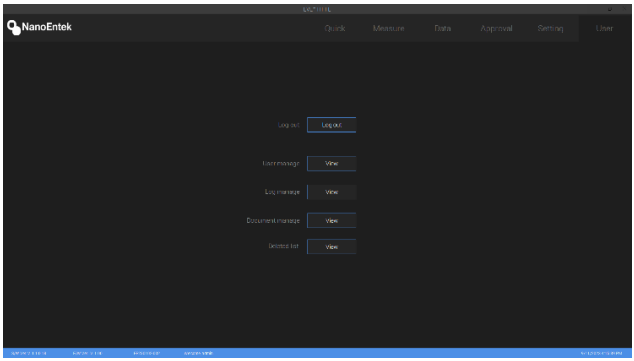
② Select the auto logout limit time.

③ Click the Auto Logout **'Setting'** button.

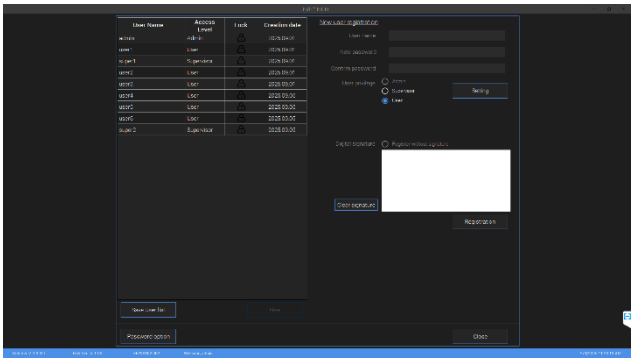
21 CFR part 11

User manage

When the 21 CFR part 11 program is activated, the user tab becomes available.



Click the User manage **'View'** button.



1. New user registration.

① Enter the user ID and PW.

② Click the User privilege **'Setting'**.

③ Set the User privilege in each menu.

④ Set the User and Supervisor permission.

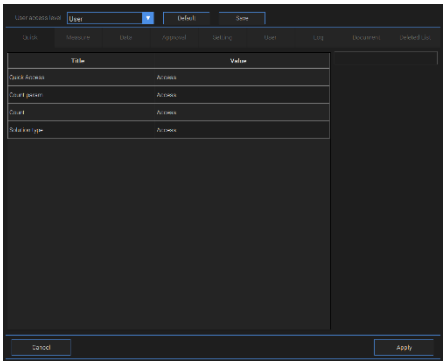
⑤ Click the **'Apply'** button.

⑥ Enter the signature and Click the **'Registration'.**

☞ ***Note**: **'Registration without signature'** is allowed, and the user will be required to register their signature at the time of first login.*

21 CFR part 11

User manage



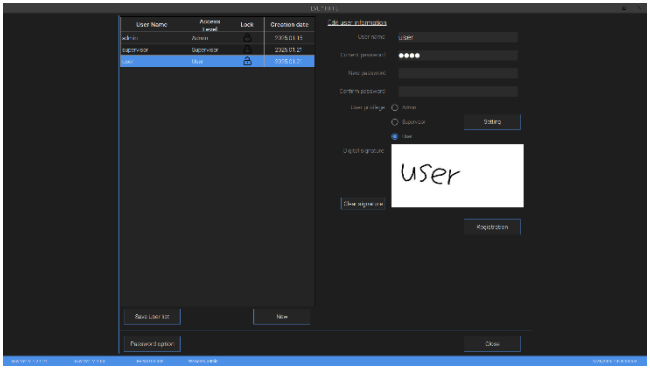
User and Supervisor default permission settings can be changed.
- Set the privilege and click the **‘save’** button.
- See the 21 CFR part 11 Supplement for default settings.

2. Edit the user option

① Select the user in user list.

② Do the same process in Creating New user.

③ Depending on the user's granted privileges, the account can be locked.

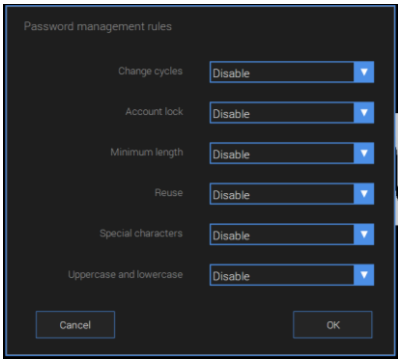


3. Password option

Set the password management rules.

21 CFR part 11

User manage



① Change cycles.
Disable, 30 days, 60 days, 180 days.
② Account lock
Disable, ≥ 3 times, ≥ 5 times, ≥ 10 times, ≥ 15 times.
③ Minimum length
Disable, ≥ 3, ≥ 5, ≥ 10, ≥ 15.
④ Reuse
Disable, ≥ 30 days, ≥ 60 days, ≥ 180 days.
⑤ Special characters
Disable, Enable.
⑥ Uppercase and lowercase
Disable, Enable.

4. Lock in user list

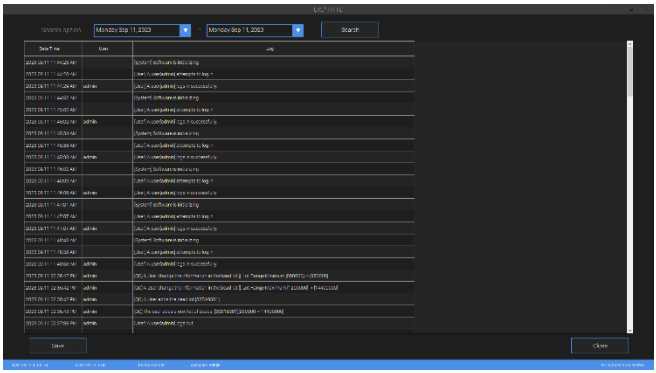
User Name	Access Level	Lock	Creation date
admin	Admin		2025.01.15
supervisor	Supervisor		2025.01.21
user	User		2025.01.21

User ID is locked when login fails. Lock icon turns red.
Click the button to unlock user ID and the button changes to grey.

21 CFR part 11

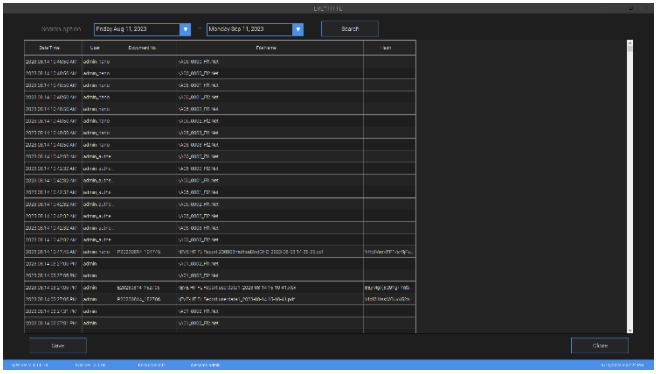
Log manage, Document manage, Deleted list

1. Log manage



This log manage allows you to view a list of instrument log.

2. Document manage

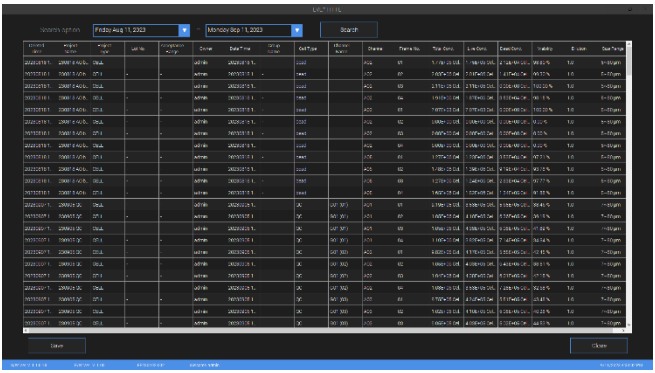


This document manage allows you to check the documents made in the equipment.

21 CFR part 11

Log manage, Document manage, Deleted list

3. Deleted list



This deleted list allows you to view a list of deleted data.

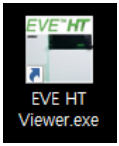
☛ ***Note**: EVE HT FL provides a comprehensive solution to comply with the requirements of 21 CFR Part 11. For more information on these features, please refer to the "Support for 21 CFR Part 11."*

Viewer program

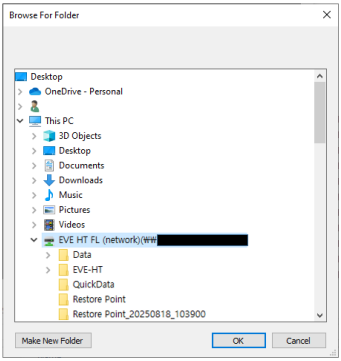
Viewer setup

EVE HT FL supports a viewer program that allows users to view, edit, and approve data on a local PC. The viewer program provides the same features as the main software, except for the functions in the Quick and Measure menus, including related settings.
Data can be accessed through a network drive connection with the EVE HT FL instrument. For this connection, the data folder must be configured for network sharing.

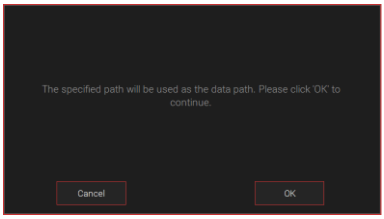
After installing the Viewer program on the local PC to be used, configure the initial settings according to the following guide:



1. Launch the EVE HT Viewer program. When the program is launched, a prompt window will appear asking you to specify the data path. Click OK to continue.



2. Specify the data path to the network folder.



3. A prompt window will appear indicating that the data path will be changed. Click OK to continue.

4. The Viewer interface will be displayed.

Maintenance and Cleaning

Clean the surface of EVE™ HT FL instrument with a damp cloth. If liquid spills on EVE™ HT FL, turn off the power immediately and wipe dry.

EVE™ HT FL does not need regular maintenance. To troubleshoot problems with EVE™ HT FL, contact technical support.

⚠ **IMPORTANT! Never disassemble or service EVE™ HT FL by yourself.**

Unauthorized repairs may damage EVE™ HT FL or alter its functionality, which will void your warranty. Contact **sales@nanoentek.com** or your local distributor to arrange for service.

⚠ **IMPORTANT! Always wipe surfaces with ethanol-soaked paper towels.**

Do not directly spray ethanol anywhere on EVE™ HT FL.

⚠ **IMPORTANT! Avoid exposing EVE™ HT FL to UV light.**

UV light may degrade components, including plastic.
Damage from UV exposure is not covered under the manufacturer's warranty.

Troubleshooting

Installation	
EVE™ HT FL does not power on	• Check on/off switch on left side of main instrument. • Check power source or contact your distributor.
Operator software does not start	• Check on/off switch on back side of main instrument. • Check connection between instrument and PC.
Inaccurate result	
Low and high results	• EVE™ HT FL is designed to read samples from 1×10^4 cells/mL to 2×10^7 cells/mL. • If your sample is out of this range, you may need to dilute the sample or add more cells and read the sample again.
Dilution factor	• Check the mixing ratio of Sample 20 µL + staining solution 20 µL. Mix ratio is already applied so do not apply to dilution factor. • Apply sample dilution to the dilution factor.
Dust or bubbles	• Check the surface of EVE™ HT FL Counting Plate. • Be careful not to make any bubbles when mixing and loading sample with a pipette. • Set the ‘Counting parameter’ before count in Focus setting window. Refer to page 19-21. • Remove any bubbles and dust in the image after count using the image edit function. Refer to page 31.
Incorrect focus	• Set the correct focus. Refer to page 18.
Too big or too many clumpy cells	• Ensure the cells are not clumped.
Inaccurate result in Bright field counting reagent	• If your sample contains small cells, Trypan Blue or Erythrosin B may not provide accurate result. Use AO/DAPI solution will improve the accuracy of the cell count. • For accurate viability determination, AO/DAPI solution is recommended.
Plate	• Push the plate all the way in.
Saving problems	
E-mail	• Check the internet connection.
USB storage	• Check the storage path.

Warranty

NanoEntek provides (1) year warranty service for defects of material and workmanship.

If any defects occur in EVE™ HT FL, NanoEntek provides repair services for the defective parts at its discretion.

The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than NanoEntek or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than NanoEntek.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For your protection, EVE™ HT FL units being returned must be insured against possible damage or loss. NanoEntek cannot be responsible for damage incurred during shipment of a defective instrument. It is recommended that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service, please contact **sales@nanoentek.com** or your local distributor.

Safety precautions

Review and follow the safety instructions below:

- If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available at your location.
- This instrument is air-cooled and its surfaces may become hot during operation. When installing, leave a space of more than 10 cm (4 inches) around the instrument and do not place any objects between the instrument and walls.
- Do not install an instrument on a slant or a place prone to vibrations, which induces the risk of malfunction or damage of the instrument.
- Never insert any objects into the air vents of the instrument as this can result in electric shock, personal injury, and equipment damage.
- Plug the power cord firmly into the wall outlet and AC adapter.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the instrument such that it is easy to disconnect.
- Turn off an instrument before unplugging the power cord and/or moving the instrument.
- If an instrument is dropped or broken, disconnect the power cord and contact a service person. The warranty will be void in case of disassembly.
- Use only authorized accessories (adaptor, power cord, and USB drive).

⚠ ***WARNING***

Class A equipment is intended for use in an industrial environment. In the documentation for the user, a statement shall be included drawing attention to the fact that there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

Mesures de sécurité

Examiner et suivre les instructions en matière de sécurité ci-dessous:

- Si de l'eau ou d'autres matières entrent dans l'instrument, l'adaptateur, ou l‘entrée de la prise, débrancher le cordon d'alimentation et contacter un technicien de service. Pour l'environnement d'exploitation, se reporter aux Spécifications du Produit.
- Ne pas toucher la prise principale ou le cordon d'alimentation avec les mains mouillées.
- S‘assurer toujours que la tension d'alimentation correspond à la tension disponible à votre localisation.
- Cet instrument est refroidi à l'air et ses surfaces peuvent devenir chaudes pendant le fonctionnement. Lors de l'installation, laisser un espace de plus de 10 cm (4 pouces) autour de l'instrument et ne placer aucun objet entre l'instrument et les murs.
- Ne pas installer d'instrument sur une pente ou un endroit sujet aux vibrations, qui entraînent un risque de défaillance ou de détérioration de l'instrument.
- Ne jamais insérer d'objets dans les évents d'air de l'instrument, car cela peut causer des chocs électriques, des blessures corporelles et des dommages de l'instrument.
- Mettre le cordon d'alimentation fermement dans la prise murale et l'adaptateur courant alternatif.
- Pour éviter tout risque de choc, s‘assurer que le cordon d'alimentation est correctement mis à la terre.
- S‘ssurer de positionner l'instrument de telle sorte qu'il soit facile à débrancher.
- Éteigner l’instrument avant de débrancher le cordon d'alimentation et/ou de le déplacer.
- En cas de chute ou de rupture d'un instrument, débrancher le cordon d'alimentation et contacter un technicien de service. La garantie sera annulée en cas de démontage.
- Utiliser uniquement les accessoires autorisés (adaptateur, cordon d'alimentation et clé USB).

⚠ *AVERTISSEMENT*

L'équipement de classe A est destiné à être utilisé dans un environnement industriel. Dans la documentation pour l'utilisateur, une déclaration doit être incluse pour attirer l'attention sur le fait qu'il peut y avoir des difficultés à assurer la compatibilité électromagnétique dans d'autres environnements, en raison de perturbations aussi bien conduisées que radiées.

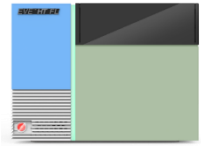
Safety Symbols

The following symbols are found on the medical device and this document. Always use the instrument in the safest possible manner.

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
	Power On/Off
	The moving parts symbol indicates areas of the medical device in which moving parts can cause injuries. Do not operate the medical device with the door open.
	This device and consumables conforms to the EC Declaration of Conformity.
	This equipment has been tested and found to comply with the limits for a Class A digital medical device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
	USB Connection
	This product conforms to UL 61010-1, CAN/CSA C22.2 No.61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements.” This instrument bearing the TÜV symbol are certified by TÜV Product Services to be in conformance with the applicable safety standard for the US and Canada.
	Catalogue number/Reference number
	Serial number
	Manufacturer
	Electrical input
	Consult Instructions for Use An electronic instructions for us (eLFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.

	Disposal of your old appliance 1. When this crossed-out wheeled bin symbol is attached to a product it means the product is covered by the European Directive 2012/19/EU. 2. All electrical and electronic products should be disposed of separately from the municipal waste stream via designated collection facilities appointed by the government or the local authorities. 3. The correct disposal of your old appliance will help prevent potential negative consequences for the environment and human health. 4. For more detailed information about disposal of your old appliance, please contact local distributor, waste disposal service or call the number listed in the manual.
	LED
	Physician. Keep dry Keep away from rain
	Fragile, handle with care
	This way up
	General symbol for recover/recyclable
	Team lift
US Corporation	US Corporation
European Corporation	European Corporation
EC REP	Authorized representative in the European community
UK Representative	Authorized representative in United Kingdom
CH REP	Authorized representative in Switzerland
BRH	Authorized representative in Brazil

Product specifications



EVE™ HT FL	
Analysis time	3 ~ 20 minutes per 48 samples
Measuring range	Detectable range: 1 x 10^4 ~ 2 x 10^7 cells/mL Optimal range: 1 x 10^5 ~ 1 x 10^7 cells/mL
Cell size range	Detectable size : 1 ~ 85 µm (Fluorescence mode) 5 ~ 85 µm (Brightfield mode) Optimal size: 5 ~ 80 µm (Fluorescence mode) 10 ~ 80 µm (Brightfield mode)
Channel	Bright field, Dual fluorescence (AO & DAPI)
Loading sample volume	20 µL per channel
Staining solution	AO/DAPI mixed solution Trypan blue solution Erythrosin B solution
21 CFR Part 11 Option	Available
Operation system	Windows 10
Power	100 ~ 240V, 50/60Hz
Dimensions	586 × 461 × 458 mm (W×D×H)
Weight	61 kg

Ordering information

Cat. No.	Description	Contents
EVE HT FL	High-throughput fluorescence cell counter	Main device 1 ea Desktop & monitor 1 set Multi pipette 1 ea
EVFL-020	EVE HT FL Counting kit	960 tests / kit Counting plate (48 channels × 20 plates) Mixing well plate (96 wells × 10 plates) Reservoir (5 pcs × 4 packs)
EVAD-960	AO/DAPI Staining Solution	20 mL × 2 bottles Acridine orange (AO) & 4',6-diamidino-2-phenylindole(DAPI) stain - Expires 2 months after opening
EVTB-960	Trypan blue Stain	20 mL × 2 bottles Trypan blue Stain solution (0.4%) - Expires 6 months after opening
EVEB-960	Erythrosin B stain	20 mL × 2 bottles Erythrosin B Stain solution (0.05%) - Expires 6 months after opening
EHGQ-001	EVE HT FL QC Plate Fluorescence (optional)	Low level, 1 pc
EHGQ-002	EVE HT FL QC Plate Fluorescence (optional)	Middle level, 1 pc
EHGQ-003	EVE HT FL QC Plate Fluorescence (optional)	High level, 1 pc
EFB-001	EVE HT FL Test Beads (optional)	1 x 1 mL / Pack

Ordering information

Cat. No.	Description	Contents
EHBQ-001	EVE HT FL QC Plate Bright (optional)	Low level, 1 pc
EHBQ-002	EVE HT FL QC Plate Bright (optional)	Middle level, 1 pc
EHBQ-003	EVE HT FL QC Plate Bright (optional)	High level, 1 pc
EHB-001	EVE HT BR Test Beads (optional)	1 x 1 mL / Pack
EHPP-001	Preparation plate (optional)	Preparation plate
EVE HT FL 21 CFR Part 11	EVE HT FL 21 CFR Part 11 software (optional)	21 CFR Part 11 software

Technical support

Visit our Website at **www.nanoentek.com** for:

- Technical resources, including manuals, FAQs, etc.
- Technical support contact information
- Additional product information and special offers

For more information or technical assistance, please call or email.

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EVE™ HT FL

NESMU-EHTFL-001E (V.0.3)

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